



Strategies for the detection of food pathogens and contaminants

Hearty, Stephen; Leonard, Paul; Sheehan, Alfredo Darmanin; Stapleton, Sharon; Tully, Elizabeth; Dunne, Lynsey; Daly, Stephen; McDonnell, Barry; Skottrup, Peter; O'Kennedy, Richard

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Strategies for the detection of food pathogens and contaminants

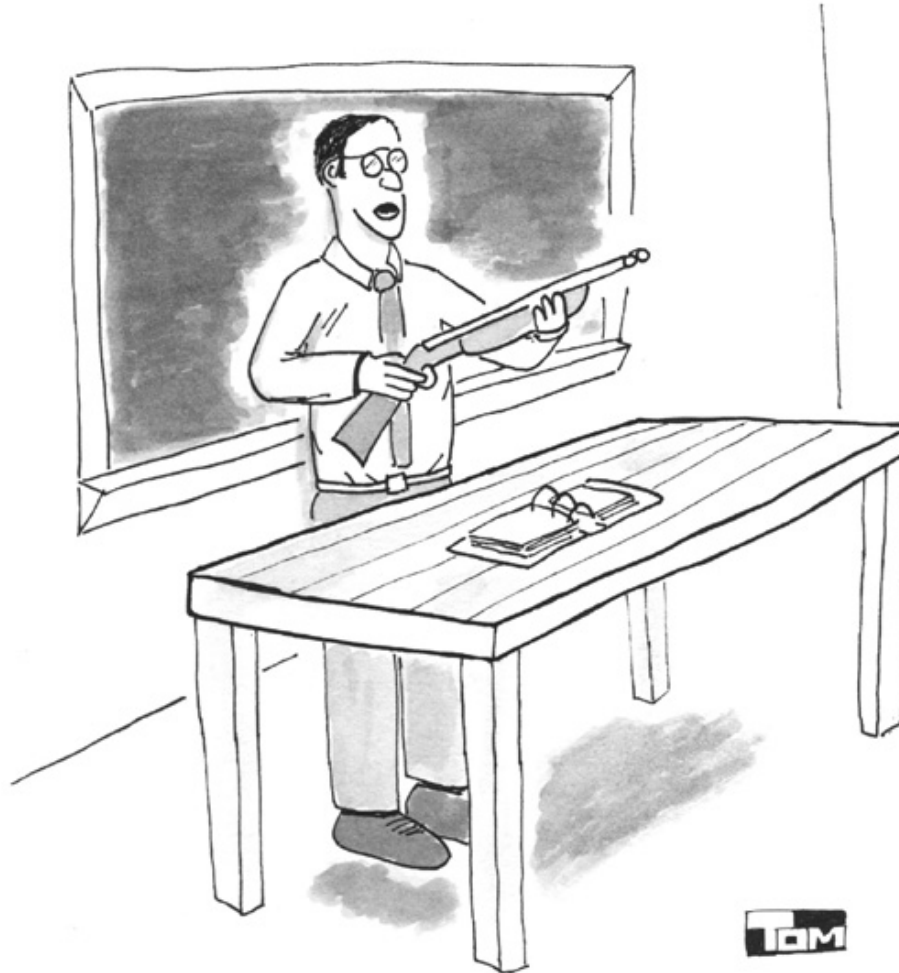
Biacore Food Analysis Symposium 2006
Kempinski Hotel, Berlin, September 27th 2006

Stephen Hearty, Paul Leonard, Alfredo Darmanin Sheehan, Sharon Stapleton, Elizabeth Tully, Lynsey Dunne, Stephen Daly, Barry McDonnell, Peter Skottrup and Richard O'Kennedy

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"PLEASE FEEL FREE TO INTERRUPT
IF YOU HAVE A QUESTION."



TOM



Biomedical Diagnostics Institute

- Established 1st Oct. 2005
- SFI CSET: Industry-Academic Partnership
- Investment: SFI €16.5m, Industry €6m

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biomedical diagnostics institute

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Biomedical Diagnostics Institute

The Biomedical Diagnostics Institute (BDI) is a multidisciplinary research institute focused on the development of next generation biomedical diagnostic devices. These devices which will directly affect the quality of peoples lives worldwide over the coming decades, will be used in Point of Care applications as well as for self-test, home use.

The BDI has developed a cutting edge collaborative research programme involving leading researchers form academic institutions, companies and the clinical environment to form a complementary, integrated, cohesive partnership.

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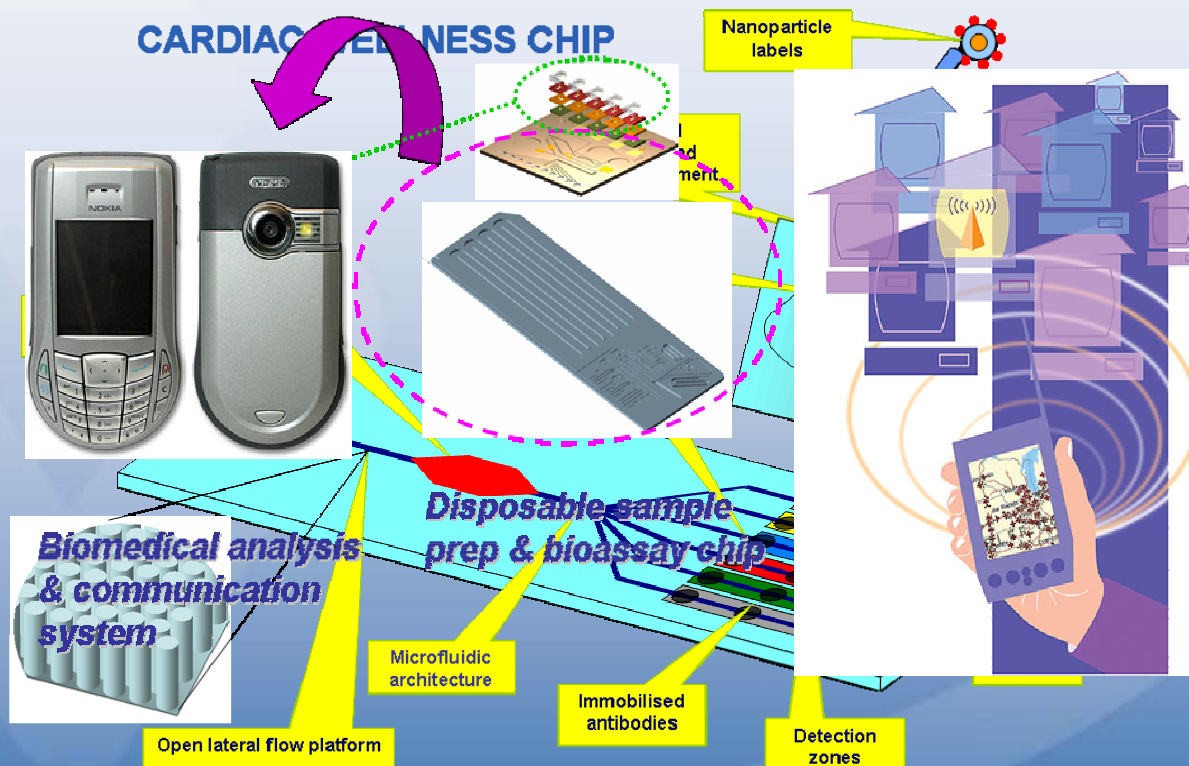




Biomedical Diagnostics Institute

• Next-generation Biomedical Diagnostics

- Early warning of life-threatening events
- Link therapy and monitoring
- POC and self testing (home use)





Biorecognition Group

...what do we do?

“....the production, optimisation and characterisation of antibodies and antibody fragments”.



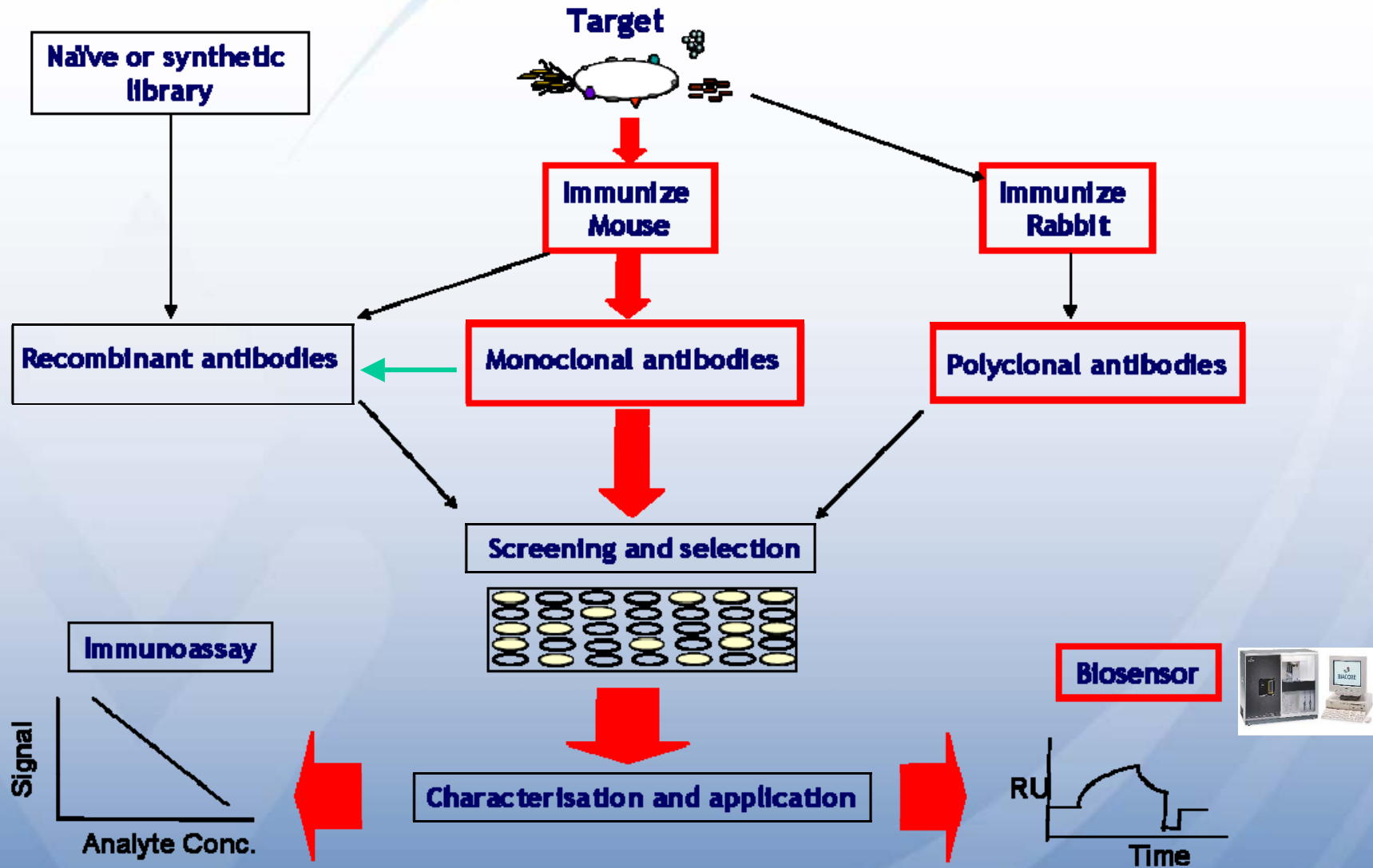


Overview

- ***Listeria monocytogenes*** (Whole cells)
 - Subtractive inhibition assay (anti-whole cell pAb)
 - Direct inhibition assay (anti-InlB pAb)
 - Direct cell binding (anti-InlA mAb)
- ***Puccinia striiformis*** (Spores)
 - Subtractive inhibition assay (anti-spore cell mAb)
- **Aflatoxin B1**
 - Inhibition assay (anti-AFB1 scFv)
- **Cephalexin**
 - Inhibition assay in milk (mutated scFv antibody)



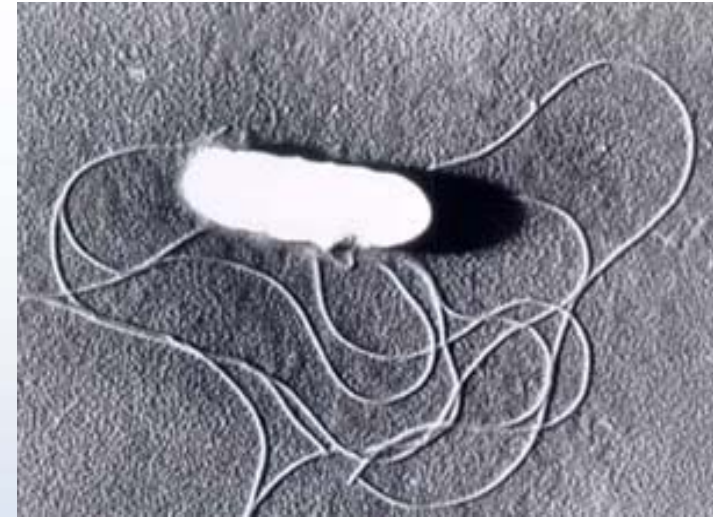
Strategy





Listeria monocytogenes

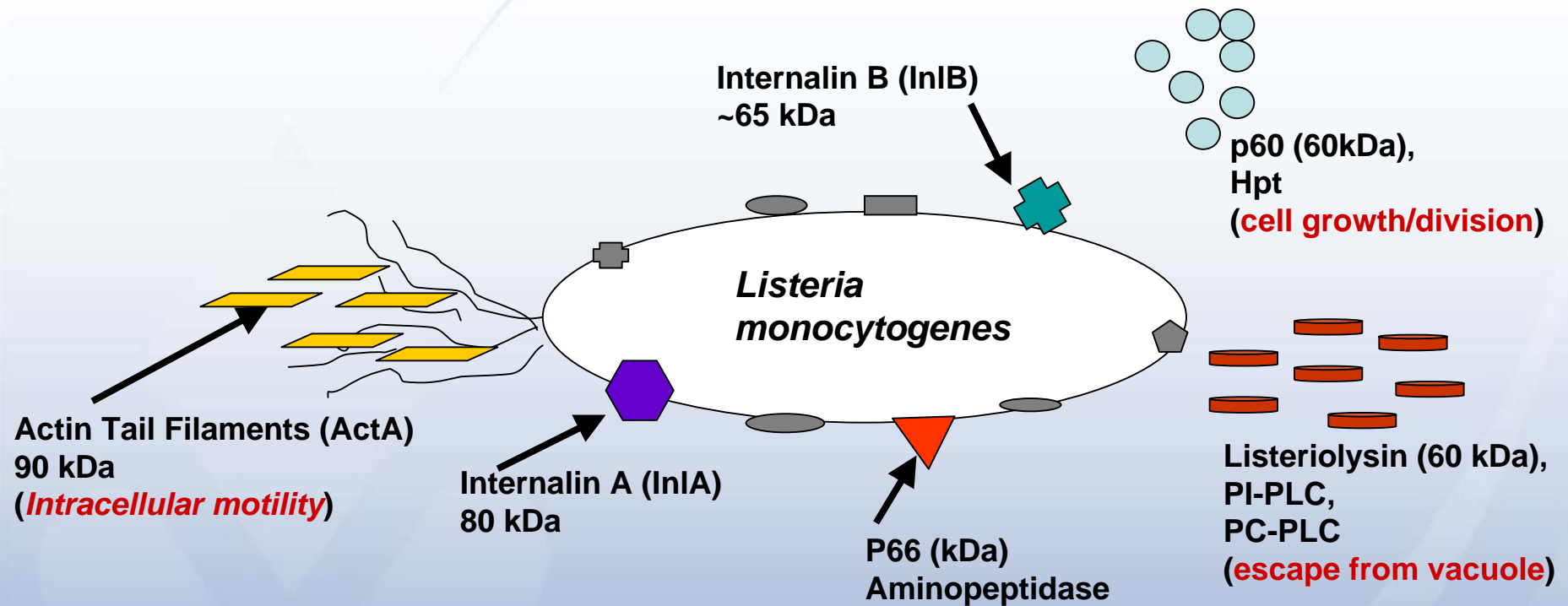
- Gram positive bacterium
- Ubiquitous in nature
- Grow at 4°C
- 'Listeriosis' manifested as
 - food poisoning
 - spontaneous abortion
 - meningitis and encephalitis
 - >20% mortality rate



<http://textbookofbacteriology.net/Listeria.html>

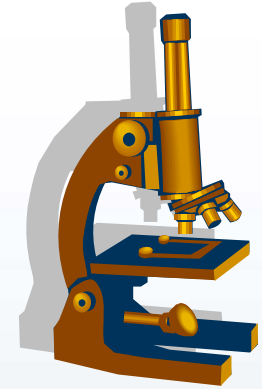


Virulence Proteins





Current Detection



First stage enrichment (24-48 hours)



Second stage enrichment (24-48 hours)



Verification (24 hours)

.....*too long!!*



Subtractive inhibition

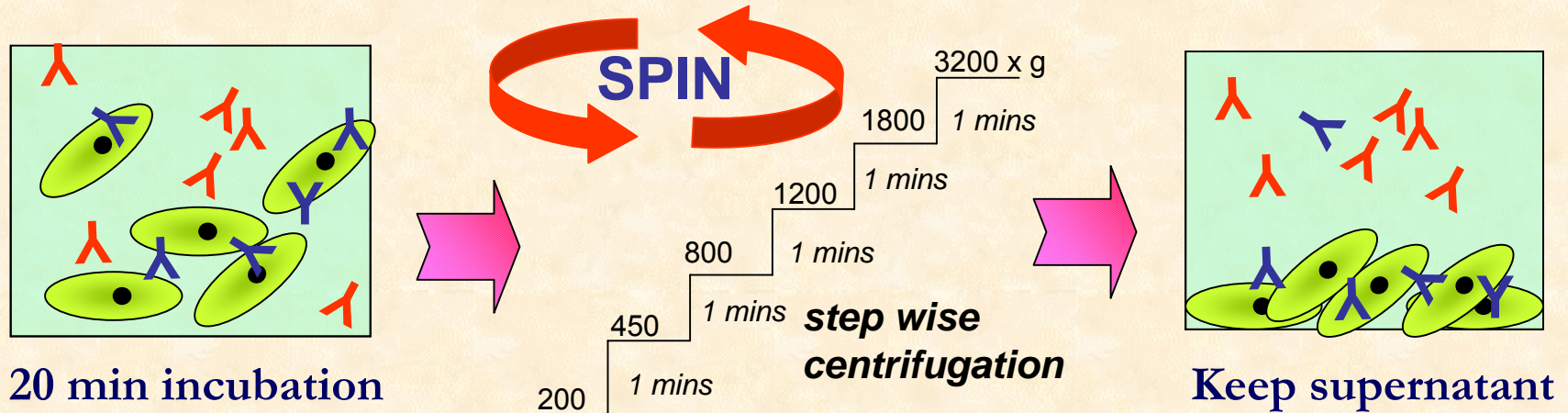
- Polyclonal antibody produced against whole cells
- Biacore assay developed:
 - Assay format
 - Preconcentration
 - Immobilisation
 - Specificity
 - Regeneration
 - Assay validation





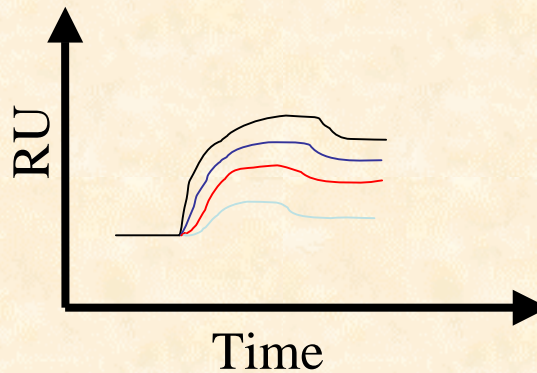
Assay: subtractive inhibition

Sample prep.

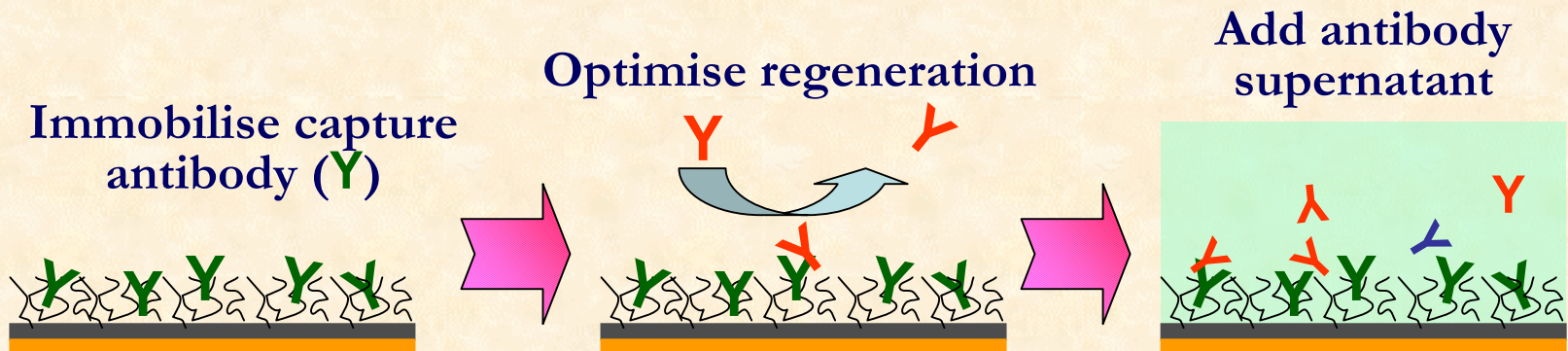


Output

Sensogram of decreasing free antibody concentrations

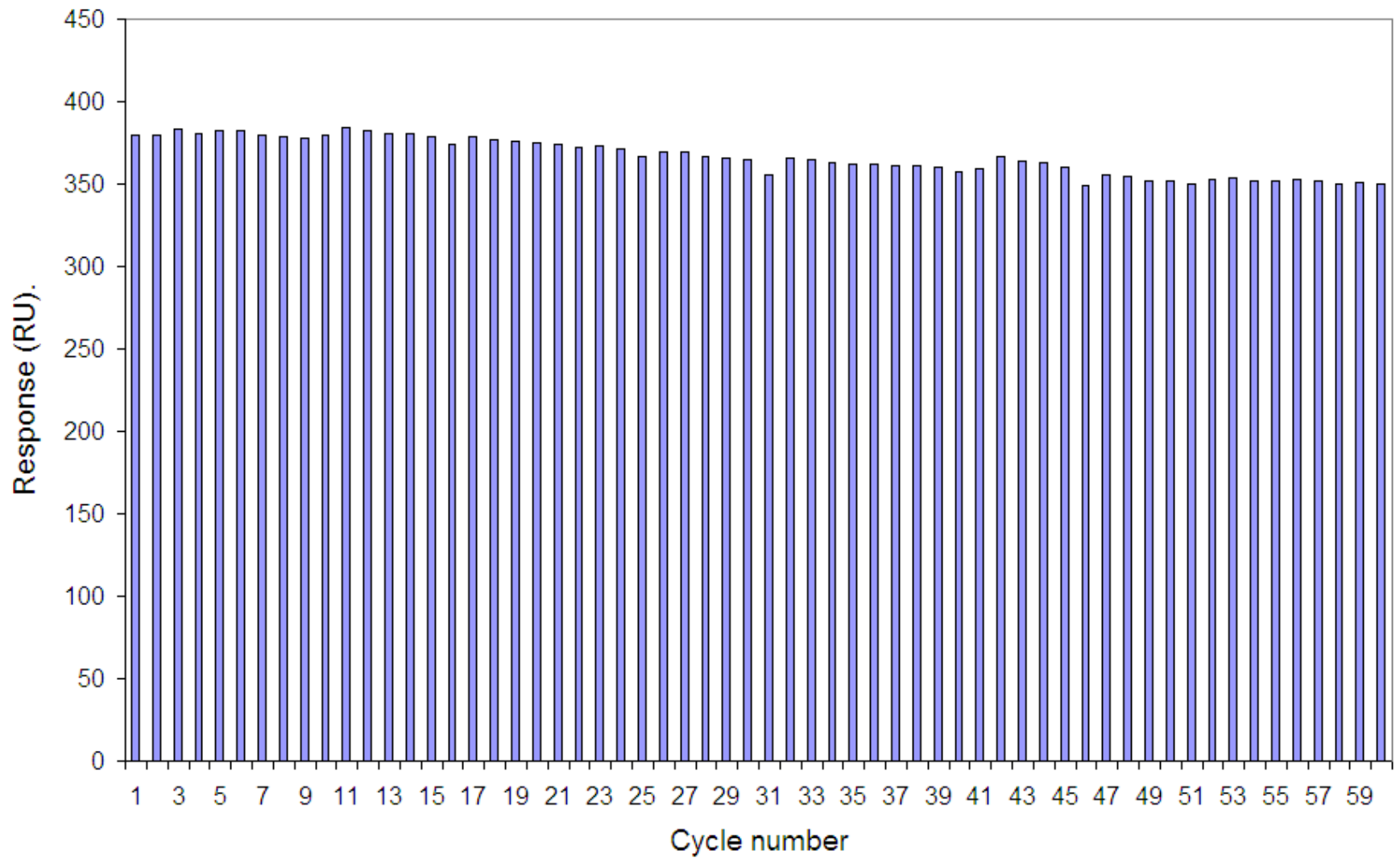


CM5 chip prep.



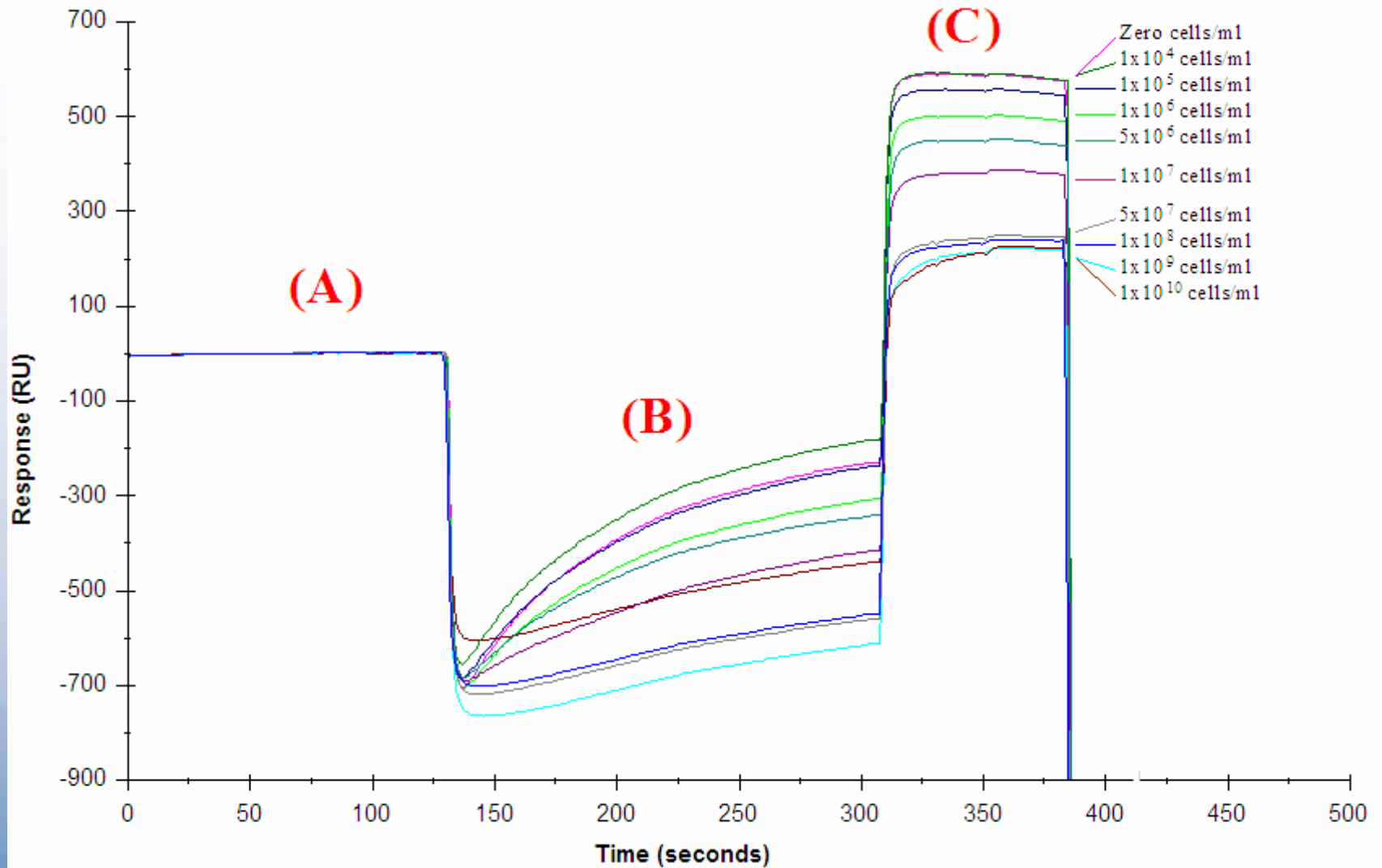


Regeneration



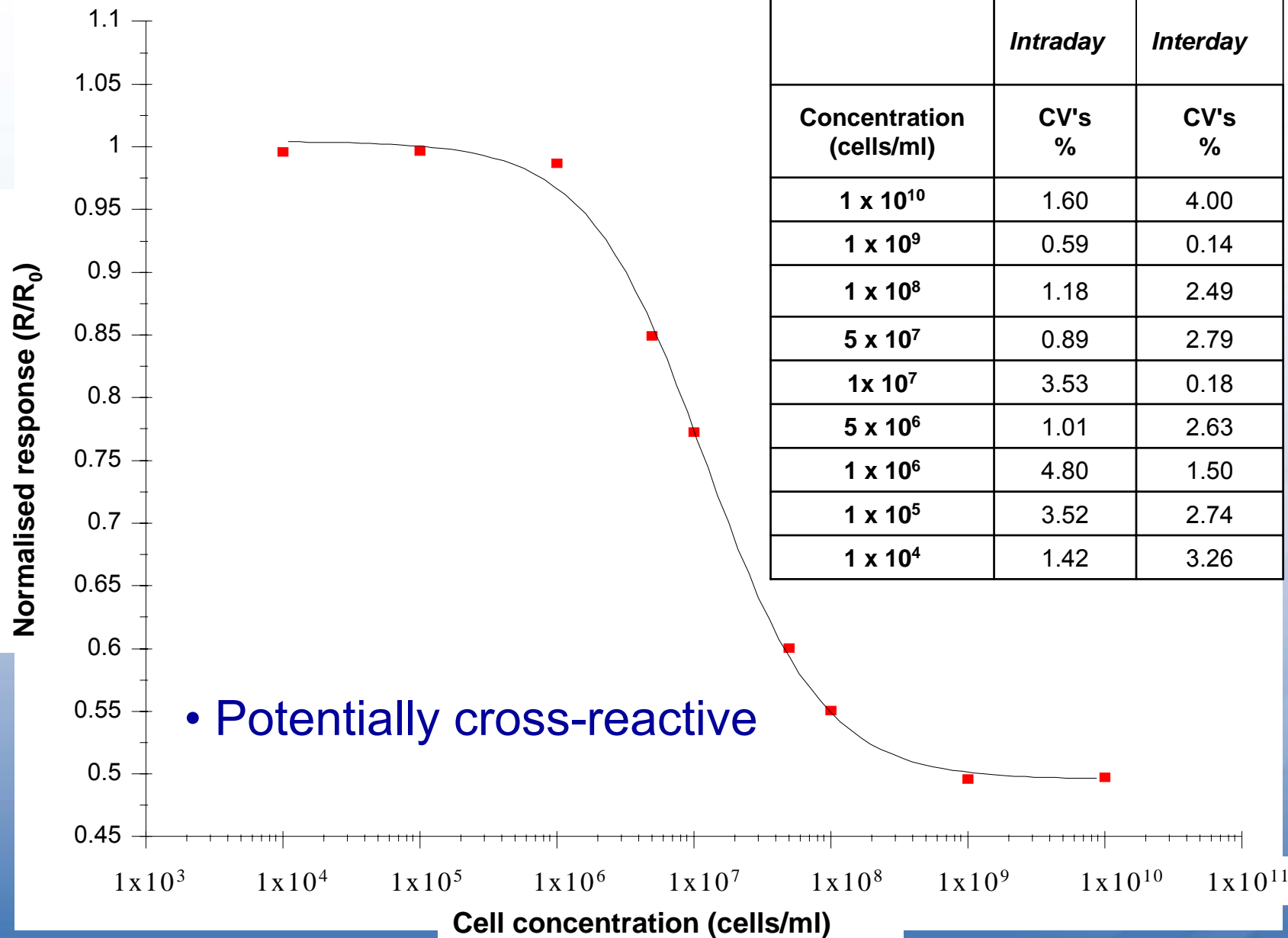


Overlay Plot





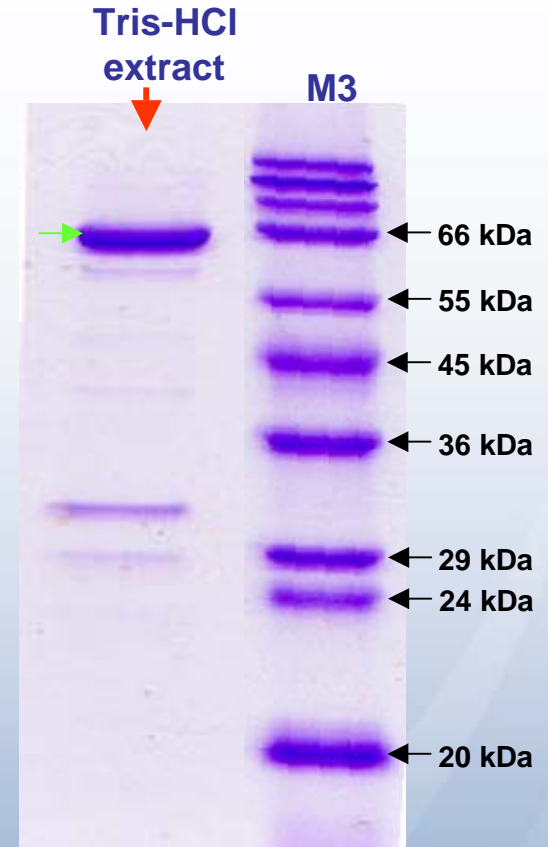
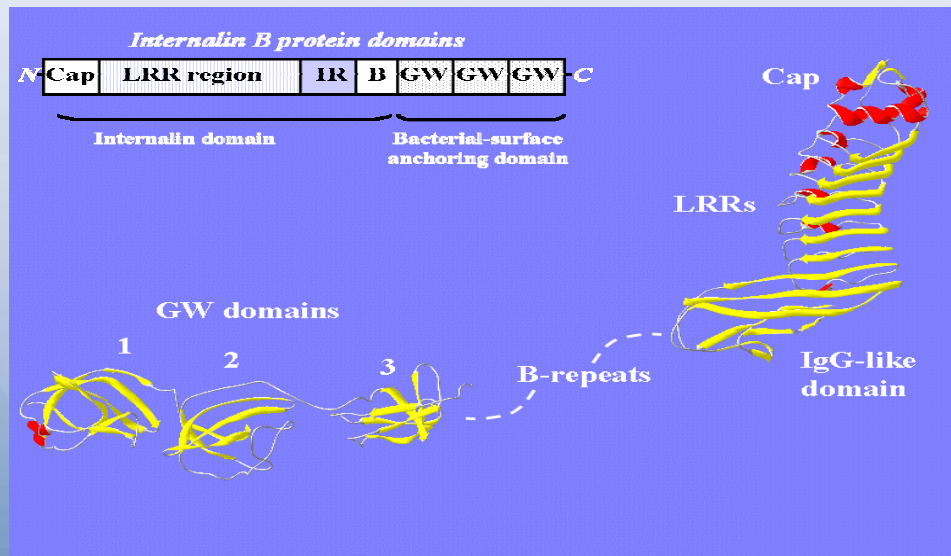
Typical Calibration Curve





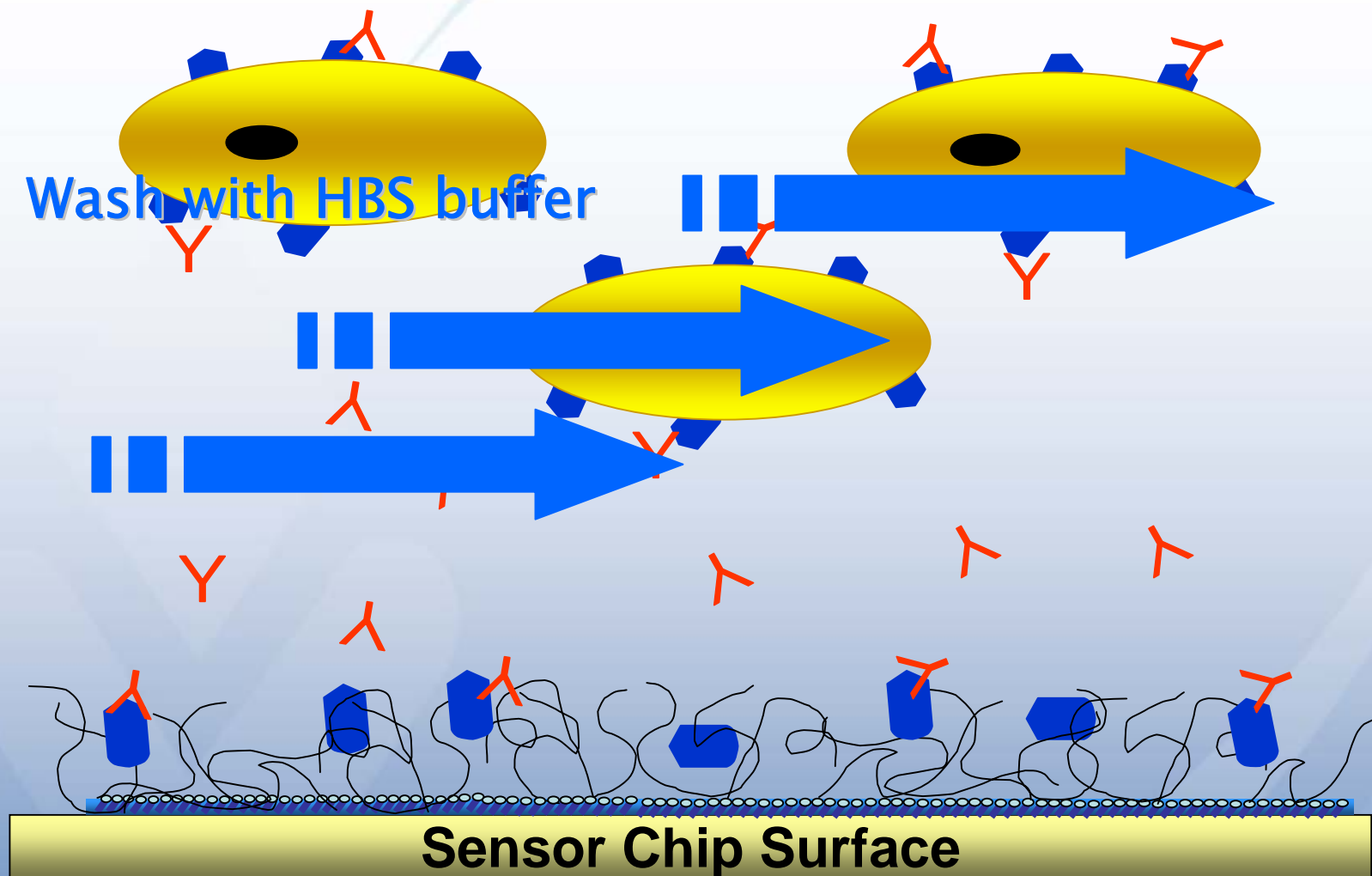
Direct Inhibition Assay

- Refine the specificity
- Anti-InIB extract polyclonal antibody
- Cloned InIB gene sequence
- Purified protein by IMAC





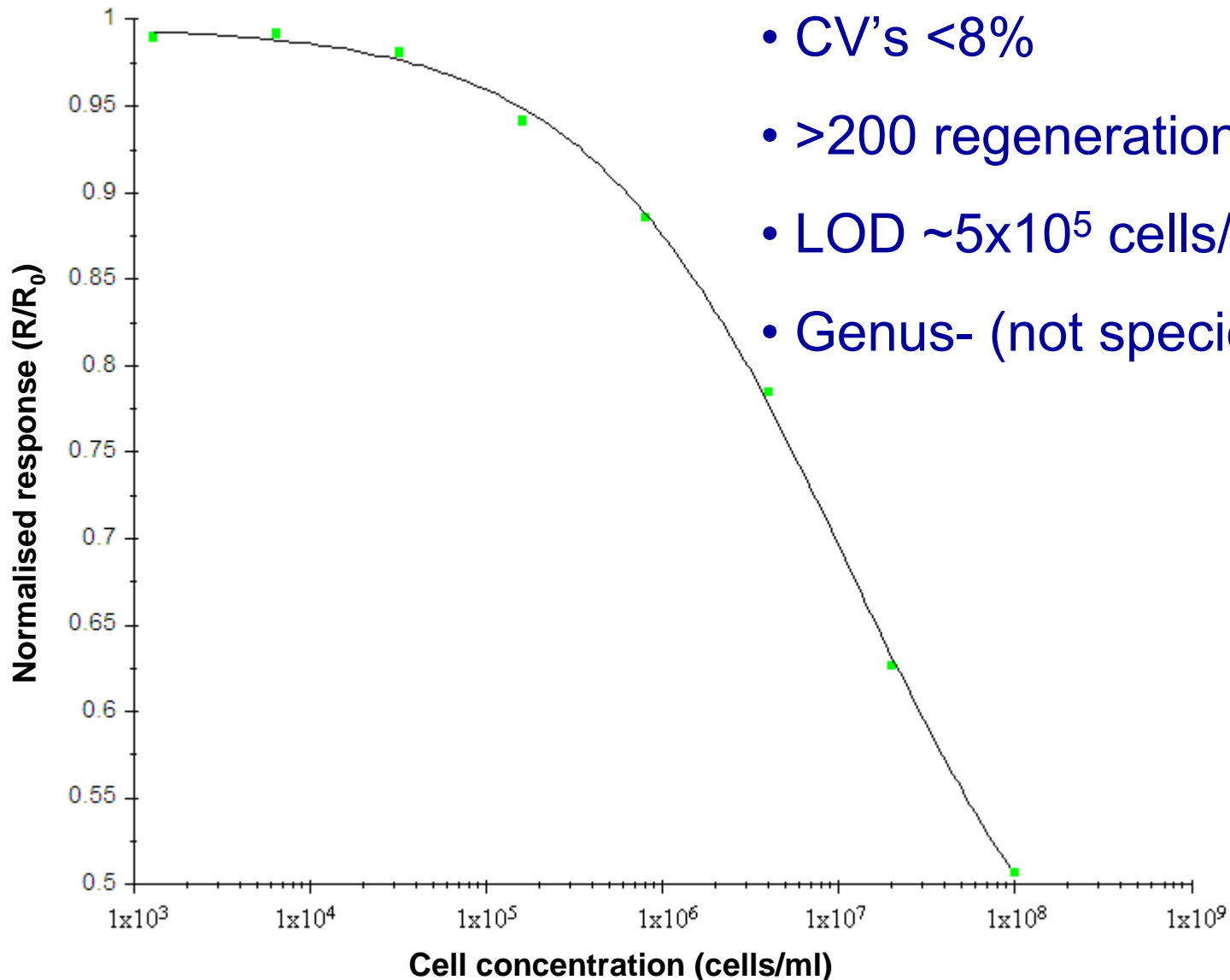
Inhibition Assay





Calibration Curve

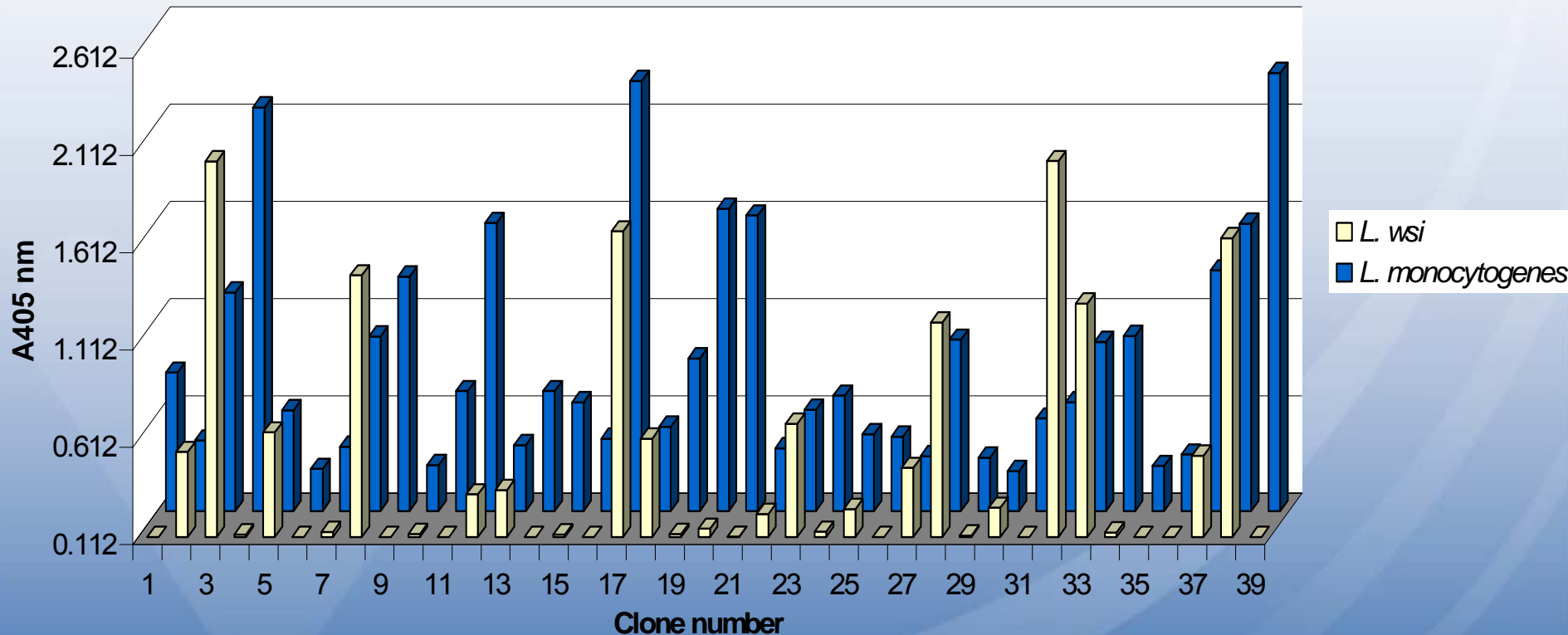
- CV's <8%
- >200 regeneration cycles
- LOD $\sim 5 \times 10^5$ cells/ml
- Genus- (not species) specific





Whole Cell Immunisation

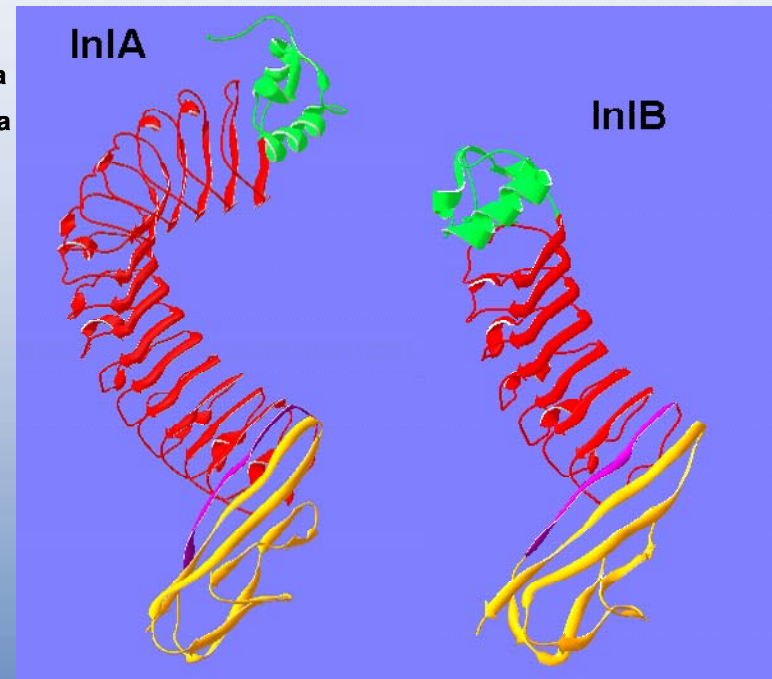
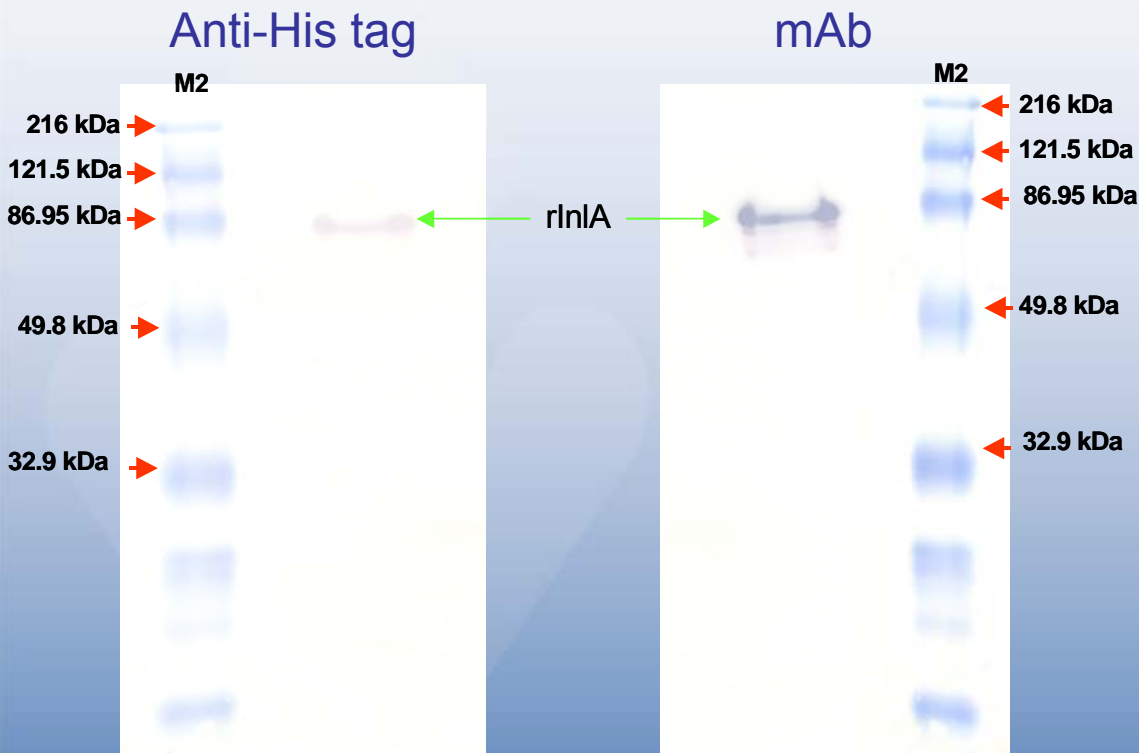
- Tandem (negative) screening
 - Putative *L. monocytogenes*-specific hybridomas
 - Selected clone no. 39 for further analysis





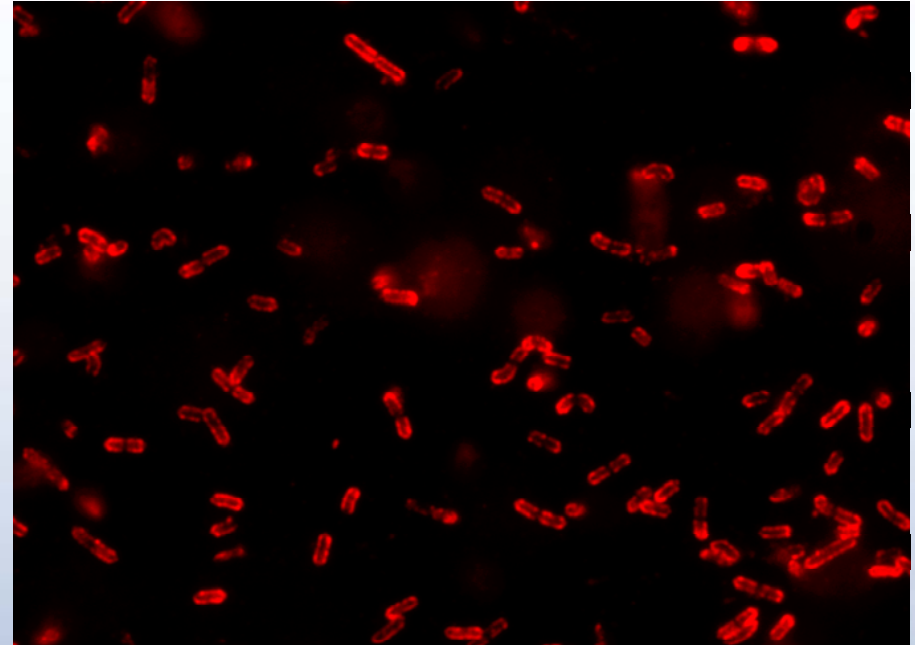
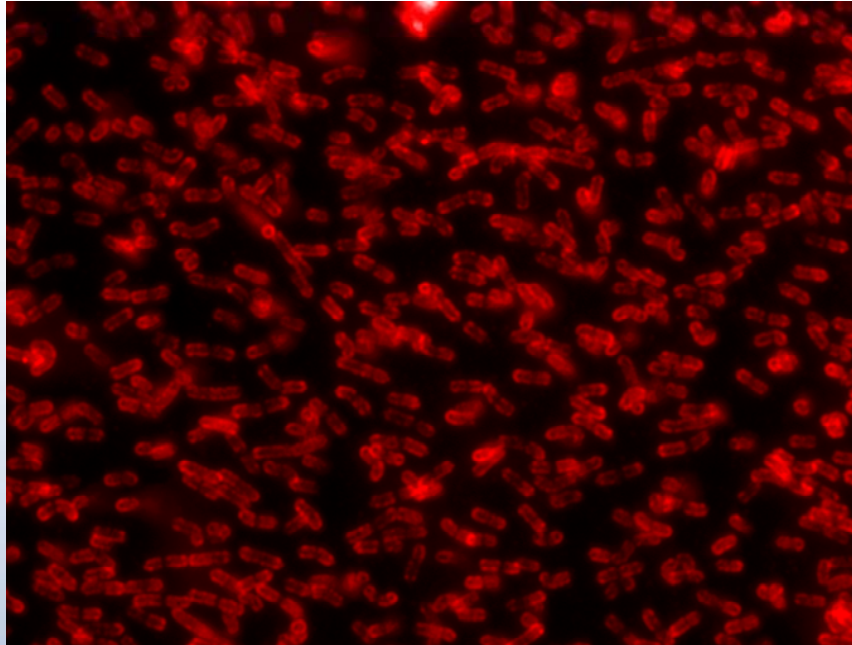
Epitope Mapping

- Confirmed specific binding of mAb to native and rInIA protein
 - Internalin A (InIA)
 - Constitutively expressed key virulence determinanant





Immuno-staining

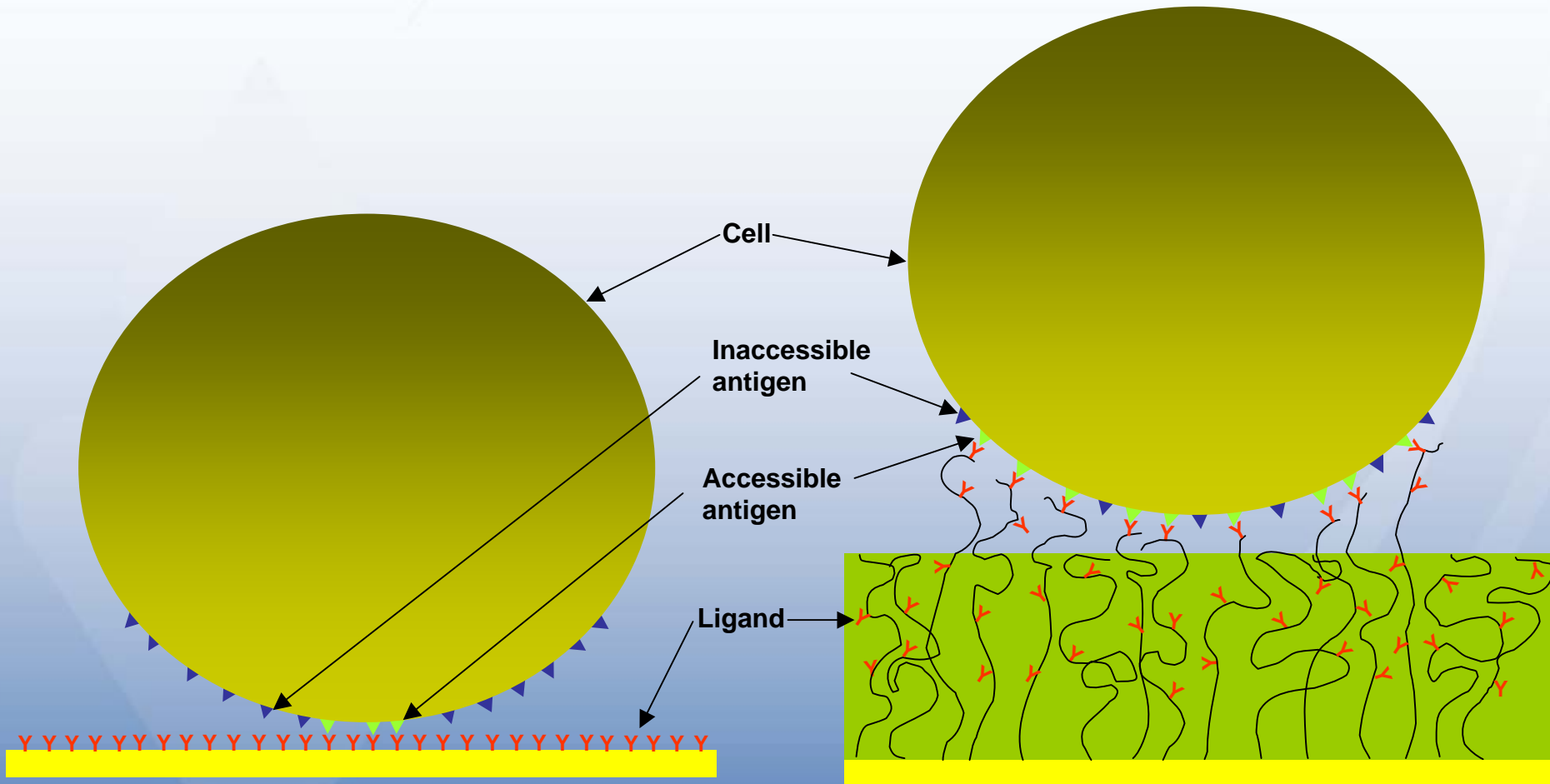


- Use of anti-InlA antibody-linked red light-emitting quantum dots (605nm), for the visualisation of *L. monocytogenes* cells on poly-l-lysine treated glass slides.



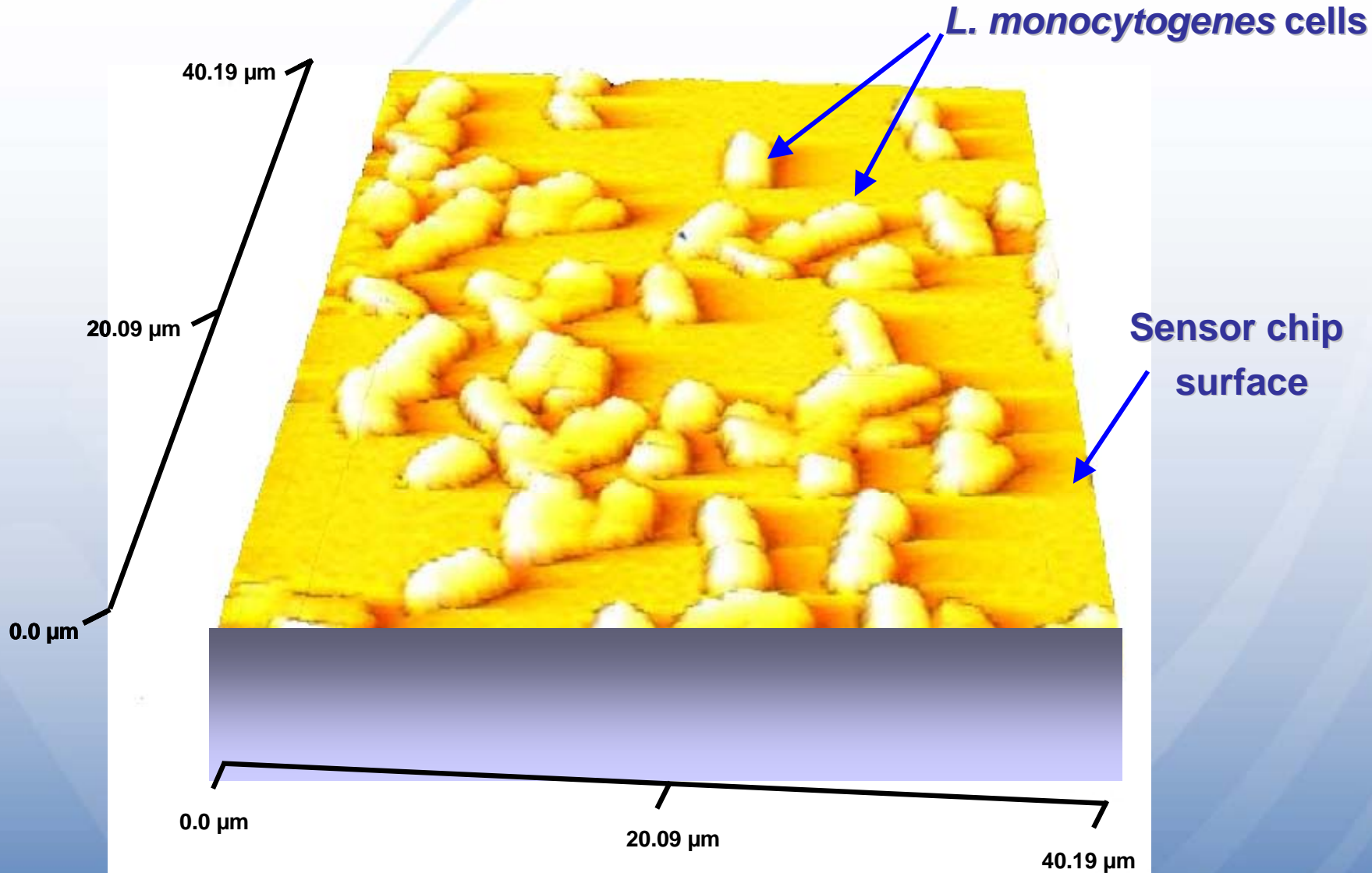
Choice of Sensor Chip

- Cell binding model





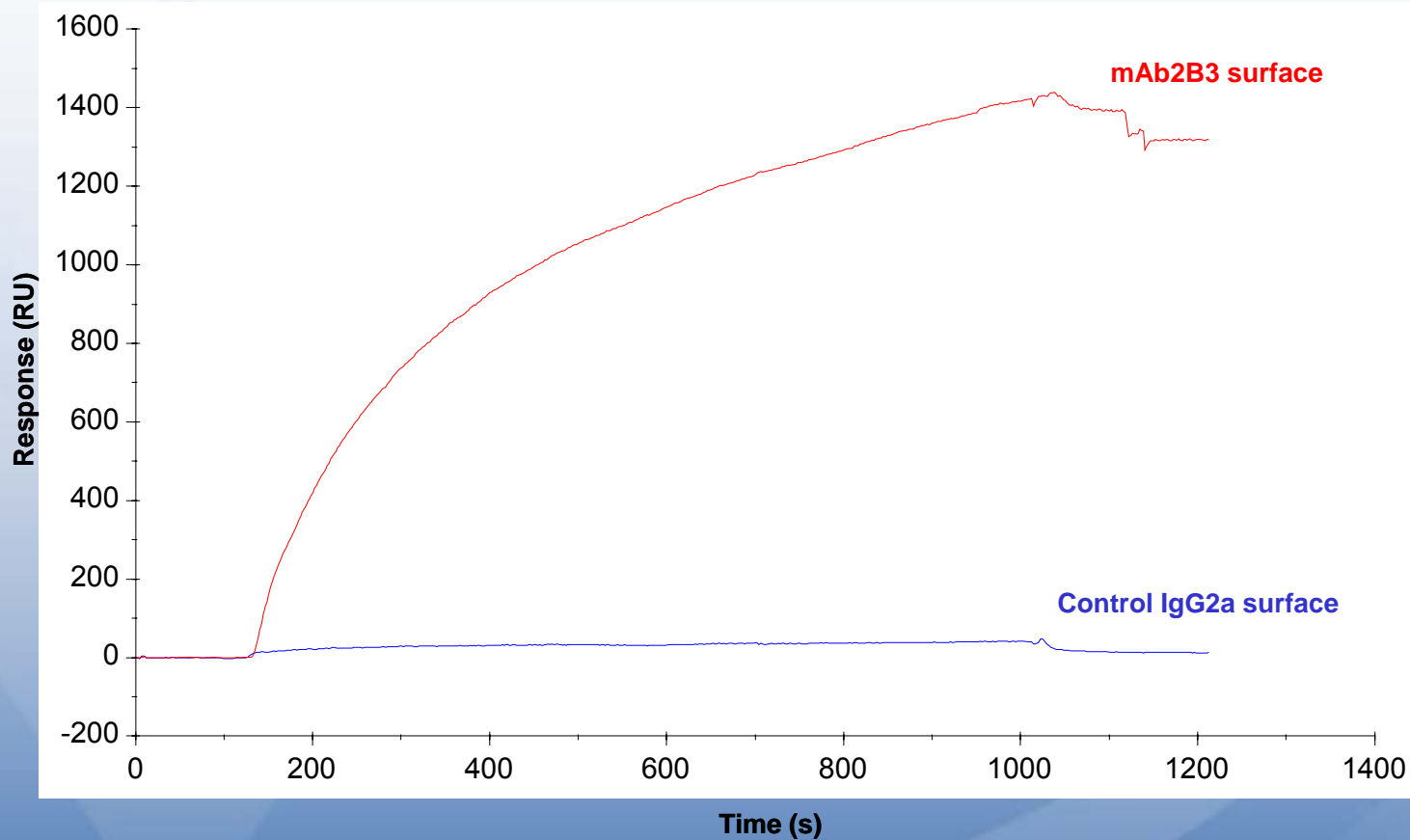
AFM Imaging





Surface Specificity

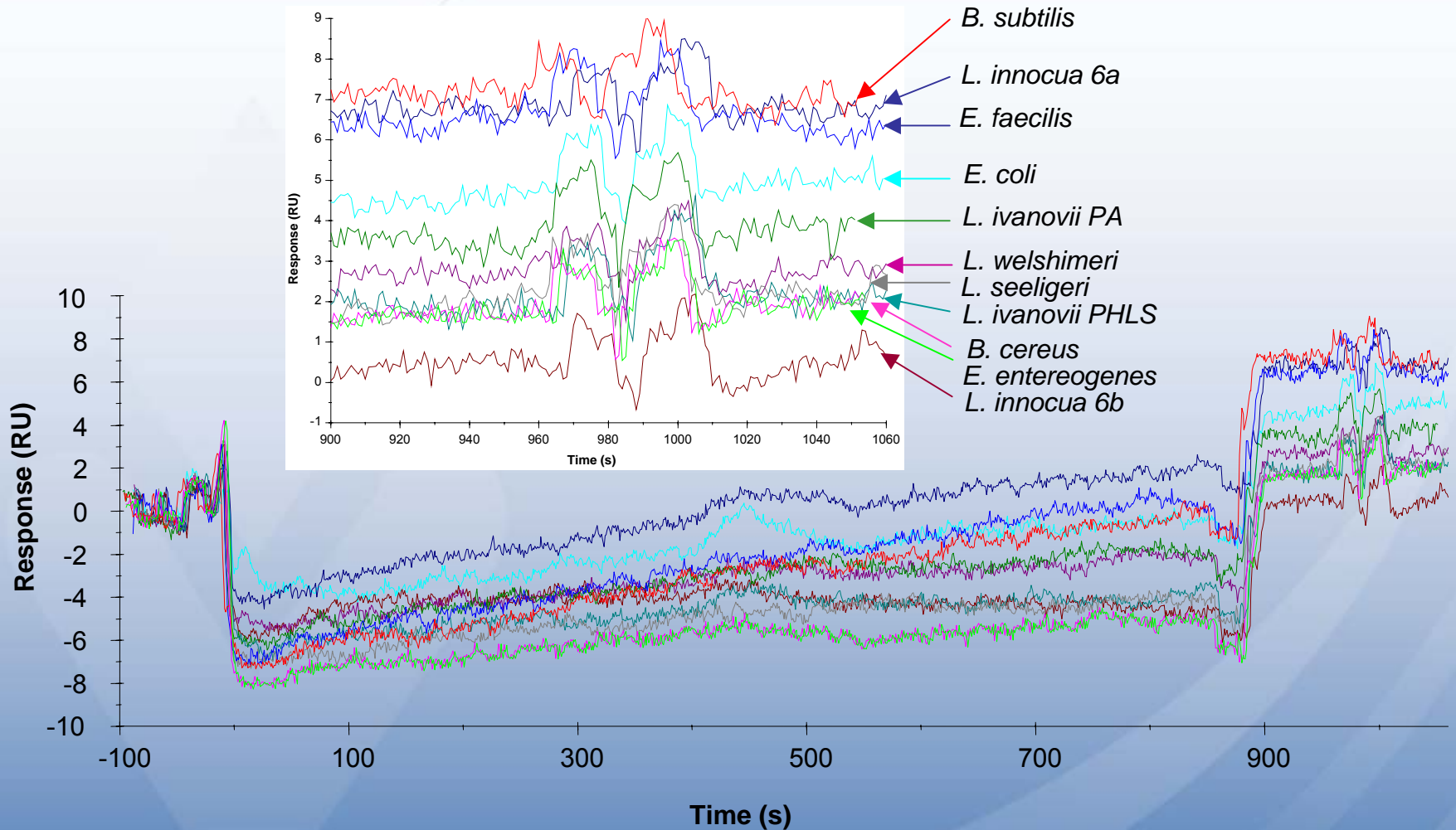
- Specific binding of *L. monocytogenes* cells to mAb immobilised surface





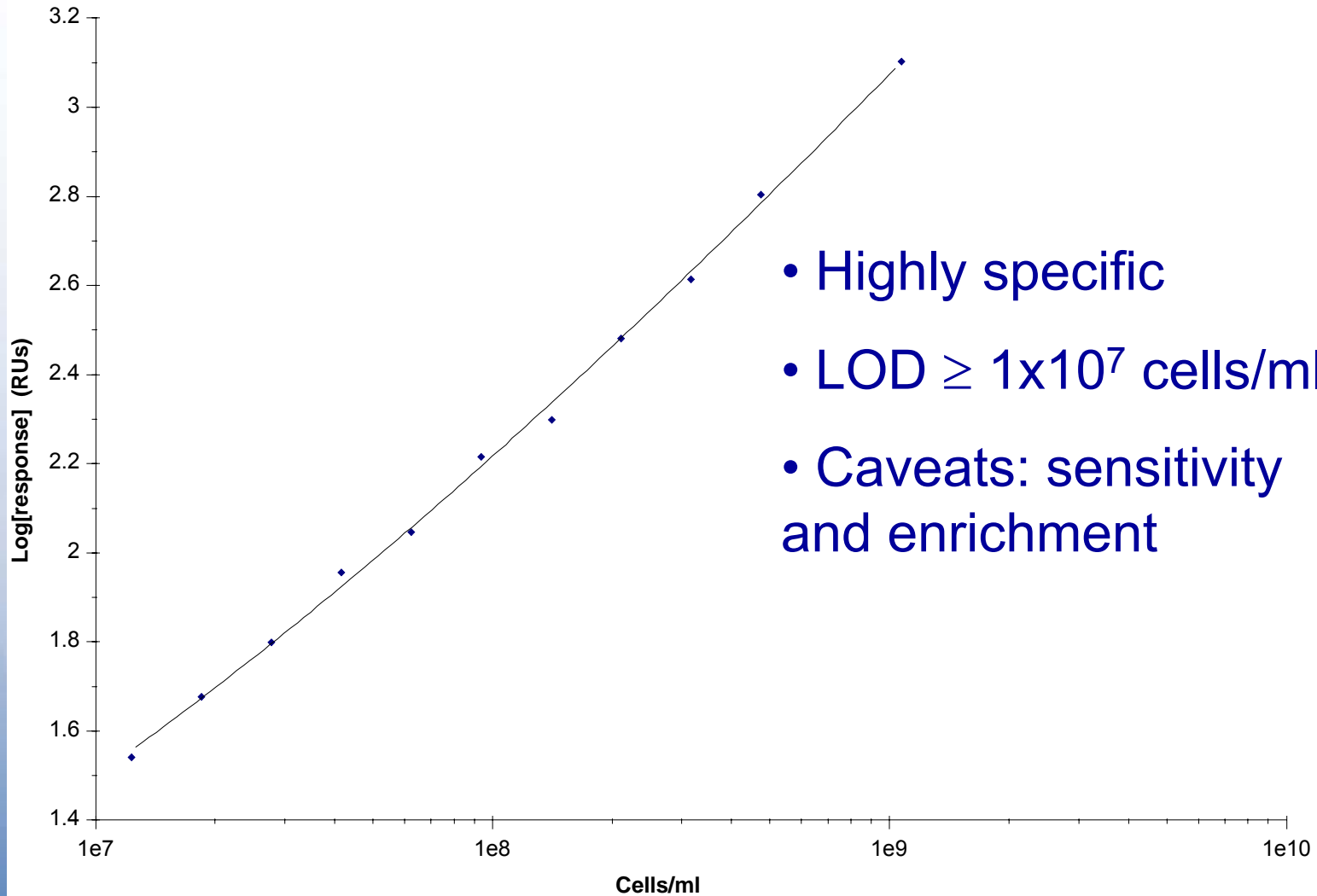
Surface Specificity

- No cross-reactivity





Typical Calibration Curve





Cereal Crop Screening

Macro-scale cereal crop farming
⇒ demand pre-harvest screening
"Precision farming"



Mapping of disease areas



Targeted application of
fungicide





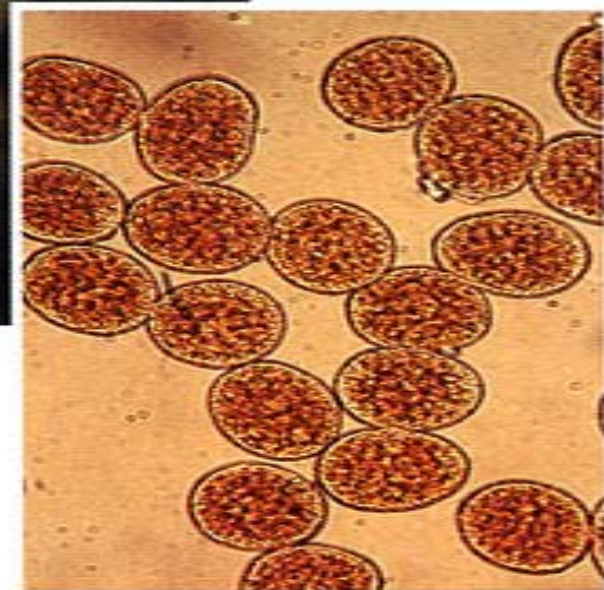
Puccinia striiformis

STRIPE RUST



Uredinia

- 18-45 μm diameter

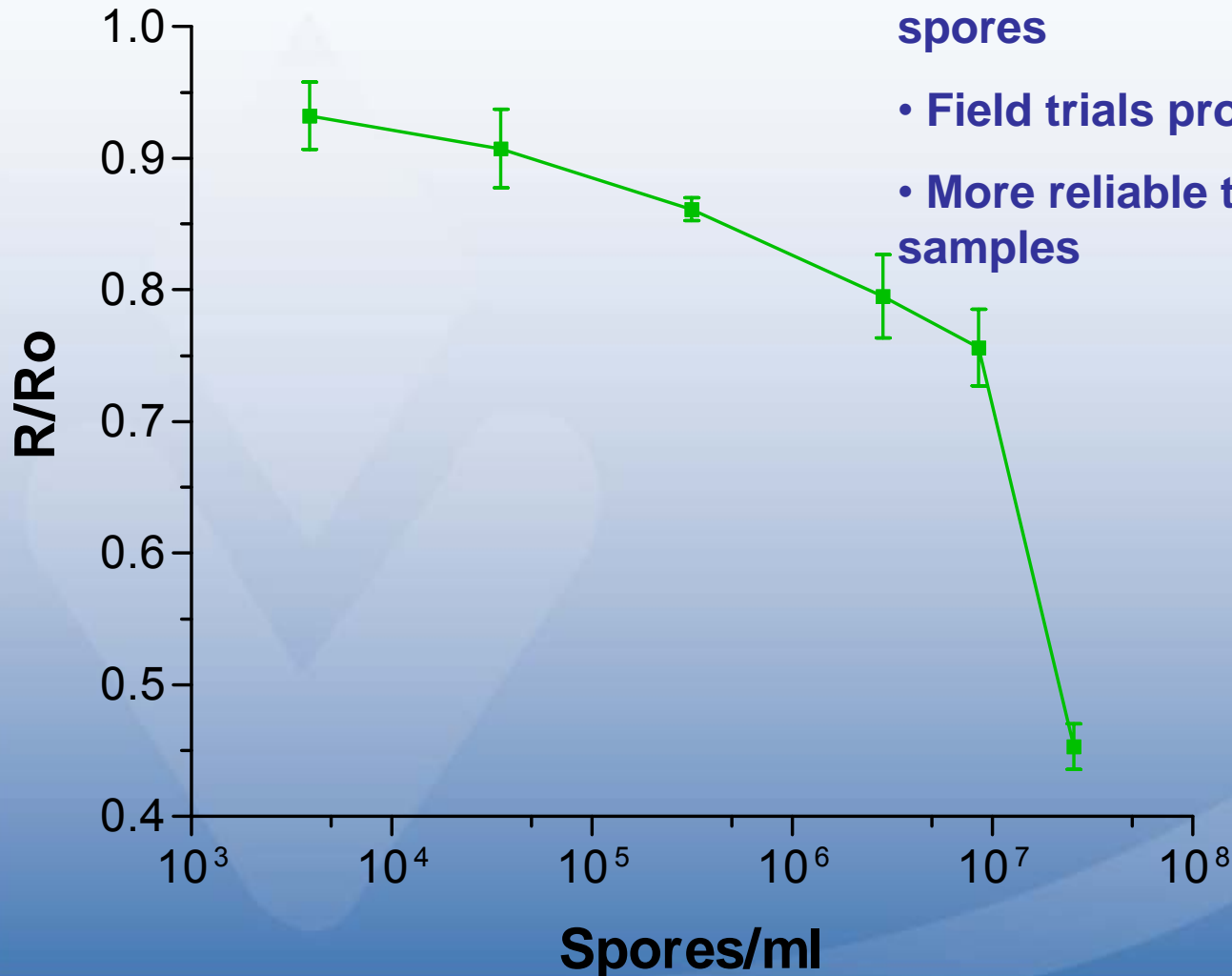


Urediniospores (400x)



Spore Calibration Curve

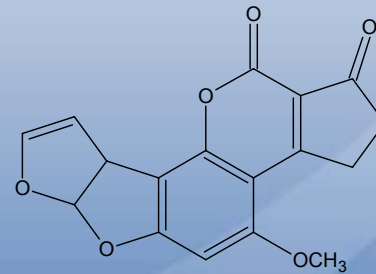
- Subtractive inhibition assay
- LOD: 3×10^5 spores/ml
- First biosensor assay reported for *Pst* spores
- Field trials proposed
- More reliable than visual analysis of leaf samples





Aflatoxins

- A group of approx. 20 related fungal metabolites that occur in *Aspergillus* species
- Most common group of toxins from naturally occurring moulds
- Aflatoxin B1 (AFB1), produced by *A. flavus* and *A. parasiticus*, is the most predominant and toxic
- AFB1 is linked to human hepatocellular cancer



AFB₁ - 312 Da



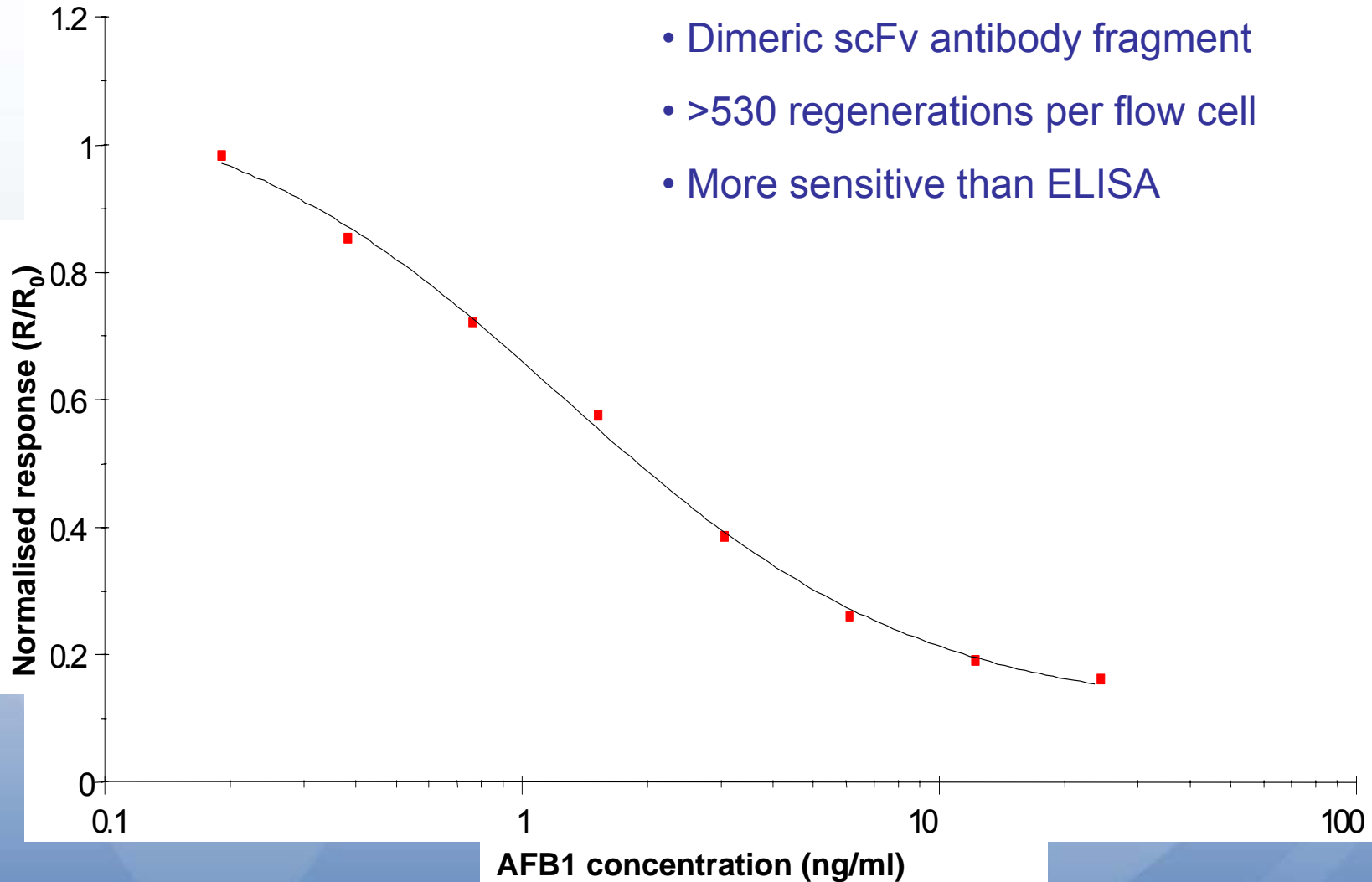
Aflatoxins

- High humidity favours fungal growth and the production of aflatoxins
- Contamination is widespread in agricultural commodities
 - ⇒ **demand for post-harvest screening**
- Acute aflatoxin poisoning in man extremely unlikely and in animals rare
- Link between dietary exposure and liver cell cancer
- EU has set maximum residue limits for aflatoxins in a variety of food types (**2-8ng/ml** for AFB1)



Calibration Curve for Aflatoxin B1

- Dimeric scFv antibody fragment
- >530 regenerations per flow cell
- More sensitive than ELISA





Biacore and ELISA IC₉₀ comparison

| Anti-AFB ₁ antibody | Competitive ELISA | Biacore inhibition assay | Improvement |
|--------------------------------|-------------------|--------------------------|-------------|
| Monomeric scFv | 12ng/ml | 0.39ng/ml | 30 fold |
| Dimeric scFv | 3ng/ml | 0.19ng/ml | 15 fold |
| Bifunctional scFv | 3ng/ml | N/A | N/A |

- Biacore can significantly out-perform ELISA



Cross-reactivity

| Aflatoxin | CR ₉₀ (%) | CR ₅₀ (%) |
|----------------|----------------------|----------------------|
| B ₁ | 100 | 100 |
| B ₂ | <1 | 1.2 |
| M ₁ | 1 | 1.5 |
| M ₂ | <1 | <1 |
| G ₁ | 10 | 20 |
| G ₂ | <1 | 1.2 |

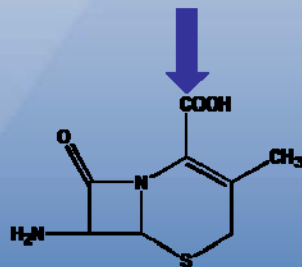
The cross reactivity potential was approximated at the least detectable dose (LDD), which is estimated at 90% R/R₀, and at the IC50 value, which is estimated at 50% R/R₀. The CR90 and CR50 were then expressed as 100-fold the ratio of the antigen and of the cross-reactant.



Cephalexin

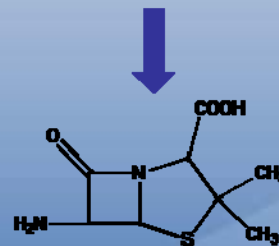
- Cephalexin is a member of the cephalosporin sub-family of antibiotics.
- Characteristic four membered β -lactam (2-azetidinone) ring
- Antibiotics used to treat livestock (e.g. Mastitis)
- Associated with an increase in body mass (i.e. sub-therapeutic dosage)
- Public health (e.g. MRSA, allergic response) and economic concerns for producers and processors

Cephalosporin Based β -lactams



7-Aminodesacetocephalosporanic
Acid

Penicillin Based β -lactams

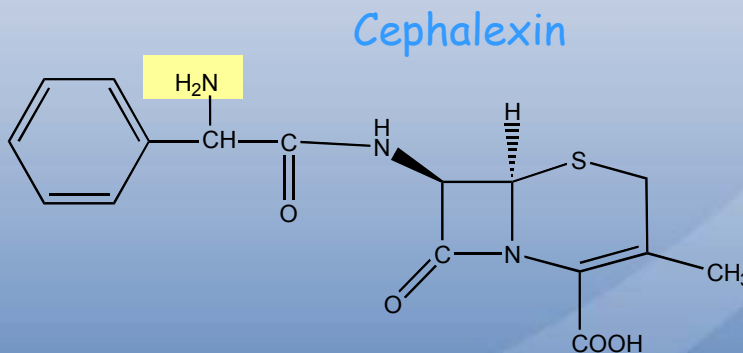


(+)-6-Aminopenicillanic Acid



Current detection

- Standard rapid assays for antimicrobial detection are based on microbial inhibition or bacterial receptor assays (e.g. Delvo SP, charm test)
- May be followed by a confirmation test (e.g. HPLC)
- Manufacturers quote limits of detection (LOD's) of 40-60 ng/ml for the Delvo SP for cephalexin
- EU Legislation
- **MRL: 100 ng/ml**





Cephalexin Inhibition Assay Format



= Thyroglobulin

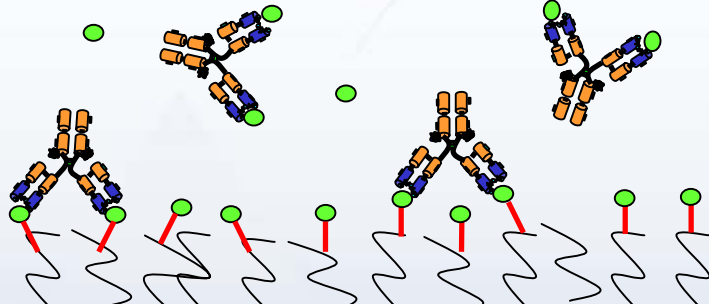


= Thyro-Ceph conjugate



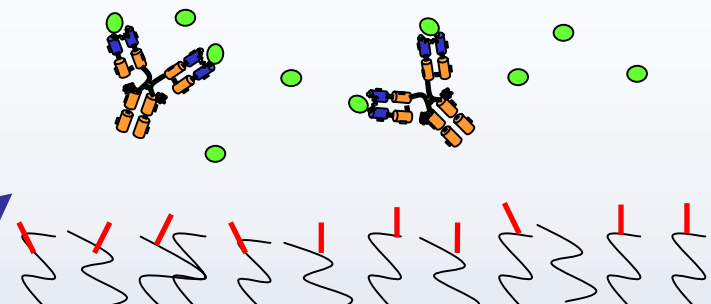
= Ethylene diamine linker

Antibody and free cephalexin injected over Cephalexin-immobilised CM5 chip surface

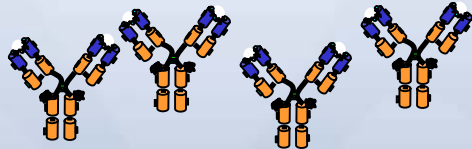


DIC- Analytical test surface

Antibody and free cephalexin injected over ethylene diamine-modified CM5 chip surface

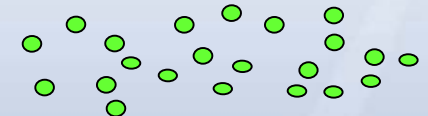


DIC- Control surface

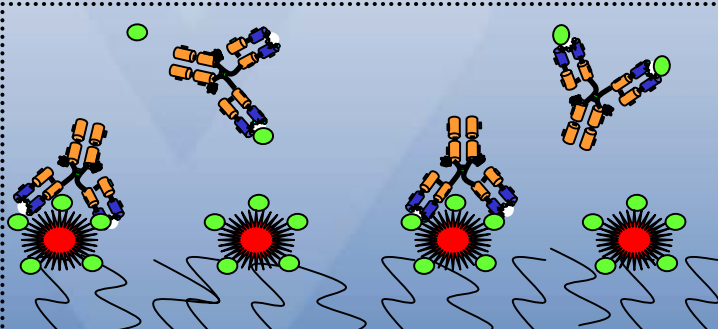


Anti-cephalexin antibody

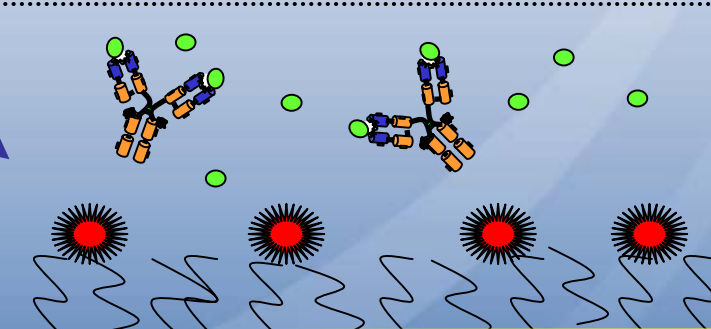
Anti-cephalexin antibody preincubated with cephalexin



Cephalexin hydrate



TCC- Analytical test surface



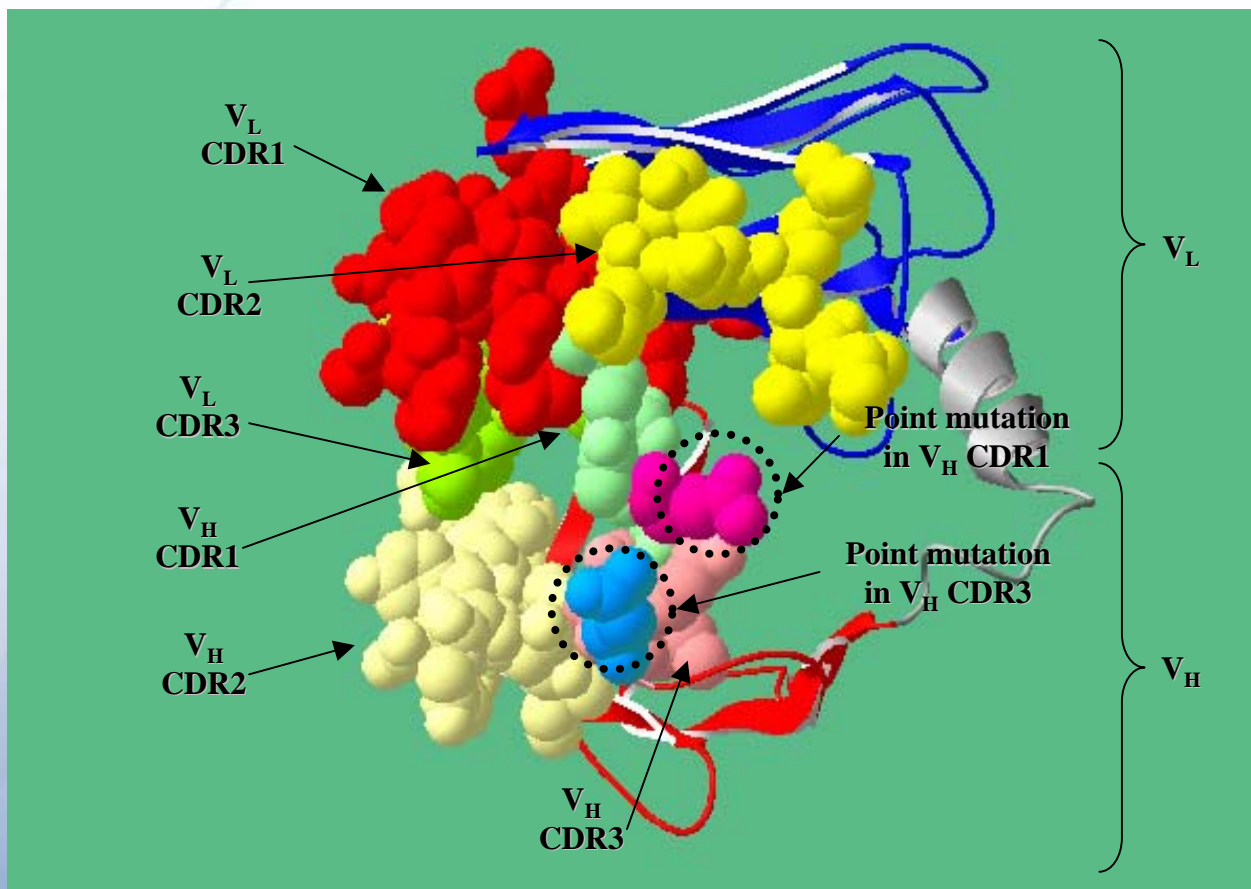
TCC- Control surface

Antibody and free cephalexin injected over Thyroglobulin-
cephalexin -immobilised CM5 chip surface

Antibody and free cephalexin injected over
Thyroglobulin-immobilised CM5 chip surface



Anti-Cephalexin scFv

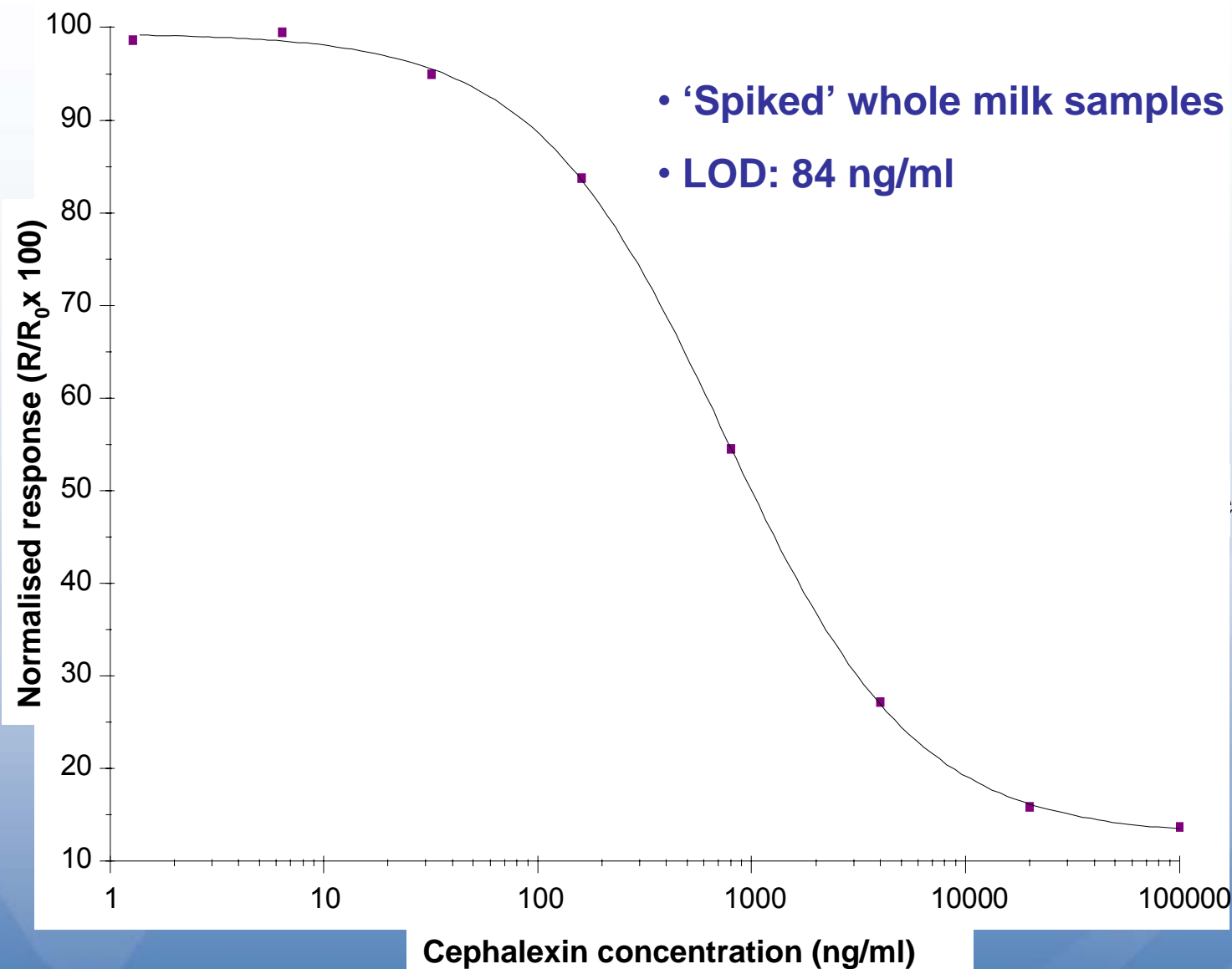


CDR H3 WT- G L G Y G K A F M D Y
C5 - G Q G Y G K A F M D Y

CDR H1 WT- S Y G M S
C5 - N Y G M S



Calibration Curve for Cephalexin





Cephalexin Assay Validation

| Response $RU/RU_0 * 100 \pm$ S.D. | Concentration of Cephalexin (ng/ml) | Back Calculated value (ng/ml) | % CV's | % Accuracies |
|---|---|-------------------------------------|-----------|-----------------|
| 13.68 ± 0.45 | 100,000 | 76,214.2 | 3.28 | 131 |
| 15.86 ± 0.94 | 20,000 | 22,256.1 | 5.94 | 90 |
| 27.14 ± 0.67 | 4,000 | 3,956.4 | 2.47 | 101 |
| 54.47 ± 0.05 | 800 | 801.5 | 0.10 | 100 |
| 83.69 ± 1.85 | 160 | 159.2 | 2.21 | 101 |
| 95.89 ± 3.21 | 32 | 33.4 | 3.35 | 96 |
| 99.43 ± 0.81 | 6.4 | --- | 0.81 | --- |
| 98.67 ± 3.45 | 1.3 | 5.9 | 2.03 | 22 |



Presentation summary

- Three *L. monocytogenes* immunoassay formats presented
- Monoclonal antibody produced that is highly specific for *L. monocytogenes*
- *Pst* spore detection demonstrated
- Biacore assay developed for the detection of Aflatoxin B1
- Aflatoxin B1 Biacore assay proved more sensitive than ELISA
- Development of a Biacore-based assay for Cephalexin using randomly mutated scFv



Acknowledgements

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Dr. Lynsey Dunne

Alfredo Sheehan

Sharon Stapleton

Elizabeth Tully

Barry McDonnell

Peter Skottrup

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Enterprise Ireland

National Centre for Sensor Research (NCSR)

InterTradeIreland Fusion

The Danish Research Council

Danish Ministry of Food, Agriculture and Fisheries



Questions





Thank You